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Congenital Syphilis in Newborn Rabbits: Immune Functions and Susceptibility to Challenge Infection at 2 and 5 Weeks of Age

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Experiments were performed to further elaborate on our congenital syphilis rabbit model. Attempts were made to determine whether in utero exposure to *Treponema pallidum* would stimulate immune reactivity and whether this activity would, in turn, affect lesion development upon challenge infection. Newborn rabbits aged 2 or 5 weeks were obtained from control does or from does infected intravenously with *T. pallidum* during pregnancy. Congenitally infected newborns exhibited increased immunologic functions. Concanavalin A-induced T-lymphocyte proliferation was elevated at both 2 and 5 weeks. In addition, macrophage Ia expression and RPR antibody titers were increased at 5 weeks. In separate experiments, newborn rabbits from control does or from does infected during pregnancy were challenged intradermally with viable organisms at either 2 or 5 weeks of age. Subsequent lesion severity was markedly increased in those newborns previously exposed to treponemes in utero. These observations further strengthen our model for congenital transmission of *T. pallidum* during pregnancy. We propose that at least some of the tissue pathology in syphilitic infection is associated with activated host defenses.

Congenital syphilis exhibits widely divergent clinical manifestations. In its most severe form, fetuses die in utero and are stillborn; in an intermediate form, the child is born with multiple birth defects; and in its mildest form, the child is born without any overt symptoms and then clinical manifestations appear 2 to 10 years later. A number of reports (4, 7, 8) have characterized early syphilitic infection in rabbits by injecting *Treponema pallidum* 1 to 6 weeks following birth. It is reasonable to assume that the resulting data have direct application to human congenital syphilis. The only drawback to this approach is that the initial treponemal exposure occurs after, rather than during, pregnancy. We have developed an animal model for congenital syphilis (5) in which pregnant rabbits are injected intravenously with high dosages of *T. pallidum* once per week over the 4 weeks of pregnancy. Two clinical outcomes are apparent. In one, fetuses die in utero or newborns die within 2 to 3 days of birth. In the second, newborns appear healthy at birth and develop normally to adult size without any overt symptoms.

The purpose of this research was to focus on those newborns that survive congenital infection and develop normally. We attempted to answer two critical questions. Are immunologic responses stimulated by in utero exposure to *T. pallidum*? If yes, do these immune responses, in turn, influence subsequent challenge infection?

The Nichols strain of *T. pallidum* was used (6). Organisms were extracted in McCoy medium supplemented with 10% heat-inactivated normal rabbit serum and 1 mM dithiothreitol. They were centrifuged at slow speed to pellet tissue debris. The supernatant was then centrifuged at high speed. The pelleted organisms were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine sera and 2×10^{-5} M mercaptoethanol. Female rabbits were impregnated and infected by using our previous protocol (5). Briefly, each pregnant doe was injected intravenously with 2×10^8 to 3×10^8 viable organisms once per week over the 4 weeks of pregnancy (days 7, 14, 21, and 28 of gestation). Newborns were sacrificed, spleens were removed, and single cell suspensions were made as described previously (16). Cells were suspended in RPMI 1640, fetal bovine sera, and mercaptoethanol and adjusted to 10^7 cells per ml. For lymphocyte proliferation, microtiter wells contained 100 μl of cells and 100 μl of medium containing 5 μg of concanavalin A (ConA) per ml (16). Suspensions were incubated for 48 h in 5% carbon dioxide at 37°C. ^{3}H-labeled thymidine (50 μl containing 1 μCi) was added for the last 18 h of incubation. For macrophage Ia quantitation, 1 ml of spleen cells was adhered for 2 h in six-well culture plates (16). After washing, adherent cells were fixed with 1% paraformaldehyde for 10 min. Two milliliters of 2% ovalbumin was added to minimize nonspecific binding. A monoclonal antibody that recognizes rabbit macrophage Ia (9) was added for 60 min (16). After washing, ^{125}I-labeled protein A was placed in each well. After 60 min, unbound radioactivity was removed by washing. One milliliter of 0.5% Triton X-100 was then added to lyse the cells and solubilize the ^{125}I activity for counting.

In the initial series of experiments, we evaluated immunologic functions at ages 2 and 5 weeks. Control newborns were compared with those exposed to *T. pallidum* in utero. Each group contained four animals. Blood was drawn, animals were sacrificed, and splenic suspensions were prepared. It was noteworthy that none of the eight infected animals exhibited overt pathology within internal organs. The spleens from these newborns, however, were slightly enlarged at 2 weeks and more prominently enlarged at 5 weeks relative to the matched controls. ConA-induced lymphocyte proliferation and adherent cell Ia expression were determined (Table 1). T-cell activity was elevated in the preparations from the newborns exposed to treponemes in utero. For the younger rabbits, the stimulation index for the controls averaged 3.3 compared with 17.9 for those infected in utero. For the older animals, T-cell activity was also enhanced in the infected preparations, but the differences

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TABLE 1. Functional parameters of spleen cells taken from newborns at 2 or 5 weeks of age

Newborn age (wk)[a]	Pregnancy treatment	ConA proliferation		Adherent cell Ia (cpm)	RPR reaction
		cpm, 10^3	Stimulation index[b]		
2	Control	6.40 ± 1.3[c]	3.3	1,165 ± 62	NR[d]
	Infected in utero	20.74 ± 1.2	17.9[e]	998 ± 76	NR
5	Control	8.57 ± 1.6	2.8	1,170 ± 49	NR
	Infected in utero	15.79 ± 1.7	5.9[e]	1,992 ± 98[e]	1:2 1:4

[a] Splenic cells were harvested and tested for functional activity at 2 or 5 weeks of age.
[b] cpm of ConA stimulated/cpm of medium alone.
[c] Standard error for four newborns for each of the four groups.
[d] NR, Nonreactive.
[e] Statistically significant relative to the controls ($P < 0.05$).

were not as dramatic. The stimulation indices were 2.8 and 5.9 for the control versus the infected in utero preparations.

The same spleen preparations were also tested for Ia expression on adherent cells as another functional indicator of immunologic activation following in utero exposure to treponemes (Table 1). At age 2 weeks, splenic cells exhibited similar levels of Ia expression in both the control and the infected in utero groups. At age 5 weeks, almost twice as much Ia was apparent in the newborns infected in utero, indicating macrophage activation.

RPR serology was performed on blood samples from each of the 16 newborns. At age 2 weeks, all sera in both the control and the infected groups were nonreactive. At age 5 weeks, the four control sera were nonreactive, whereas the four infected sera were reactive at 1:2 and 1:4 dilutions.

Attempts were then made to determine whether this enhanced immune reactivity would influence challenge infection. Newborn rabbits from does infected during pregnancy were compared with newborns from control does. In separate sets of experiments, newborns were challenged dermally with 10^5 viable treponemes. Findings are summarized in Table 2. Previous work by Gamboa and Miller (7) showed that newborn rabbits up to 4 to 5 weeks of age exhibited minimal infections; lesions were smaller in size, failed to ulcerate, and healed more quickly than their adult counterparts. In our experiments, 2-week-old newborns from normal does developed atypical lesions, and clinical manifestations averaged only 5.0 days in duration. In contrast, newborns previously exposed to *T. pallidum* in utero exhibited a shorter incubation period and more intensified lesions that averaged 12.0 days in duration. These infections were larger and more indurated. Representative lesions were aspirated and examined for motile spirochetes. All samples tested contained one to five motile treponemes per high-powered field.

Similar findings were apparent with 5-week-old newborns. More intense infections occurred in rabbits previously exposed to *T. pallidum* during pregnancy. These lesions lasted an average of 38.6 days contrasted to 13.7 days for the control newborns. Note also that better lesion development was apparent in the older control animals relative to the younger control animals, confirming the previous observations of Gamboa and Miller (7). Dark-field samples of these lesions were also positive for motile spirochetes.

These results indicate that in utero exposure to *T. pallidum* effectively stimulated immune functions in newborn rabbits. Splenic T lymphocytes exhibited increased mitogenicity to ConA at 2 and 5 weeks postbirth. In addition, splenic adherent cells had higher basal levels of Ia class II antigens at age 5 weeks, indicating in vivo activation of macrophage function. Low levels of RPR antibodies were detected only in those newborns exposed to *T. pallidum* in utero. Importantly, these antibodies were apparent at 5 weeks but not at 2 weeks of age, demonstrating that they were of newborn origin. The in vivo activation of T cells and macrophages, together with these RPR antibodies and the overt splenomegaly, strongly suggests an active infection at 5 weeks of age. This is a critical point. In our initial report of this congenital model (5) we showed that virulent treponemes were present in splenic tissue 1 week postbirth. Thus, the organisms had penetrated the maternal fetal barrier. An unanswered key question was whether these organisms were able to induce subsequent clinical infection. The results in this paper appear to answer this question.

Newborn rabbits have diminished immunologic capabilities. In the first few weeks of life, rabbit macrophages are somewhat deficient in processing and killing of different microorganisms (1, 12–15, 19, 20). In other studies we have shown that rabbit spleen cells from normal 2-week-old newborns exhibited partially decreased macrophage as well

TABLE 2. Enhancement of lesion serverity by in utero exposure to *T. pallidum* following dermal infection at 2 and 5 weeks of age

Newborn age (wk)[a]	Pregnancy treatment	EI (day)[b]	Sites positive/sites infected (%)	Duration of lesions (days)	Range (days)
2	Control	12.0 ± 2.7[c]	6/12 (50)	5.0	3–8
	Infected in utero	6.8 ± 0.3	8/12 (67)	12.0	6–19
5	Control	10.3 ± 1.7	7/16 (44)	13.7	3–35
	Infected in utero	7.9 ± 0.2	13/16 (81)	38.6	8–54

[a] Newborns were infected intradermally with 10^5 treponemes at 2 or 5 weeks of age.
[b] Initial day of erythema and induration (incubation period).
[c] Standard error for six to eight newborns for each age group.

as T- and B-lymphocyte functions (17). Macrophage interleukin-1 and Ia were decreased, T-lymphocyte interleukin-2 and gamma interferon and proliferation were diminished, and B-lymphocyte proliferation was decreased. Given these lowered immune capabilities, it might be anticipated that infection of newborns would be more intense since treponemes should be able to vigorously multiply unimpeded by activated host defenses. However, just the opposite occurs. Gamboa and Miller (7) infected newborns at various times postbirth. Lesions developed poorly when animals were infected dermally between ages 5 days and 4 to 5 weeks. Our observations relate to these. In utero exposure to *T. pallidum* activated macrophage and T-lymphocyte functions. Upon challenge of the newborns, we anticipated even poorer lesion development due to faster immune-mediated clearance. Again, however, the opposite occurred and lesion intensity was increased rather than diminished.

To explain these strange findings, we suggest that immune-mediated damage directly contributes to lesion pathology in newborns. This concept was initially suggested by Turner and Hollander (18) with adult rabbits. When animals were infected dermally and given daily cortisone, lesion pathology was significantly delayed. Upon termination of cortisone therapy, lesion severity greatly intensified within 1 week (rebound phenomena). In similar experiments, Lukehart et al. (10) evaluated the histopathology within lesion tissue. Cortisone delayed cellular infiltration; termination of cortisone then resulted in marked T-cell and macrophage infiltration, leading to increased pathology. These cortisone observations point to tissue damage associated with immunologic activation. This may apply to our findings in this paper. One other immunologic component may also contribute to tissue damage. Rheumatoid factor (immune complexes) has been found in the sera of human patients with congenital syphilis (2, 3, 11). This factor could contribute to histopathology by enhancing the intensity of vasculitis. Future efforts will focus on immune-mediated damage elicited by macrophage secretion of tumor necrosis factor and interleukin-1, both of which can damage host tissues.

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